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SUBJECT OF INVESTIGATION

STUDIES ON THE MECHANISM OF CELL DAMAGES IN LIVER AND KIDNEY CELLS AND IN HEART MUSCLE FIBERS AS REVEALED BY ELECTRON MICROSCOPY

RESPONSIBLE INVESTIGATOR

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1. The purpose of the investigation

In this research project we would like to study the changes of cell organellae in case of various cell injuries caused by various noxe, the mechanisms of toxic effects of which are known or yet unknown, such as chemicals, cyto-toxin, bacterial toxin, etc., mainly by electron microscope. The light optical histochemistry and electronmicroscopic histochemistry will be applied also when necessary. The results which will be obtained will help much in clarifying the mechanisms of cell degeneration (cloudy swelling, hydropic degeneration, fatty metamorphosis, necrobiosis, necrosis, etc.) and finally may contribute in preventing such damages.

2. The preliminary experiments already done:

a. Acute poisoning of Monofluoracetate in rat.

(1) Material and method.

Natrium salt of monofluoracetae was administered in dose of 5 mg/kg. body weight (0.025 % solution in physiological saline) to the rats (185-250 gm.). Liver was examined electron microscopically 10 and 50 minutes after intraperitoneal injection of monofluoracetate. Usual osmic acid was used as fixative and methacrylate for embedding.

(2) Results.

Marked changes were observed in mitochondria and other cell organellae beginning already 10 minutes after administration. They consist of swelling, crystolysis, and decrease in density of ground substance of mitochondria, localized dilatation of rough surfaced endoplasmic reticulum (REr), loss of RNA granules from REr and its transformation to smooth surfaced endoplasmic reticulum (SEr), decrease in number of REr, loss of glycogen, increase in number of microbody, etc. The changes of mitochondria might be interpreted as morphological expression of inhibition of TCA cycle, but changes of Er and other structures need further studies.

b. Acute intoxication of 2-4-Dinitrophenol in rat.

(1) Material and method.

2-4-Dinitrophenol was administered 100 mg/kg body weight (group A), 50 mg/kg body weight (Group B), subcutaneously as a NAOH solution having a pH.8.8 in rats (130-200 gm), respectively. The liver was examined electron microsconically 15, 30, 50 minutes after injection in Group A, 3, 6, 12, and 24 hours after injection in Group B. Usual osmic acid was used as fixative, and epoxy resin or styrene for embedding.

(2) Results.

(a) Group A. After 15 minutes, there are localized dilatation of Disse's space filled with red blood cells, mitochondria, glycogen granules, ground substance of cytoplasm, etc., of destructed liver cells. Bile canaliculi are generally dilated. There are two types of liver cells. One is characterized by somewhat darker cytoplasm filled with seemingly swollen mitochondria. The other is cells appearing as if atrophied. Their mitochondria have electron dense ground substance. Cytoplasm of this type of cells shows decreased electron density. Although endoplasmic reticulum (REr) is relatively well preserved, occasional transformation of REr to SEr by drop off of RNA granules is observed. In liver examined 30 minutes after administration of drug, there are swelling, increase of electron density of ground substance, dilatation of pale layer of crista, etc., of mitochondria, irregular vacuoles in cytoplasm, and drop off of RNA granules of REr. The nucleus shows often marked nucleolonema formation. Structures corresponding to focal cytoplasmic degradation (Spargo) are also encountered occasionally. After 50 minutes, there are similar changes together with appearance of ring made up of REr. Glycogen begins to diminish after 30 minutes.

(b) Group B.

After 6 hours, besides the changes mentioned above, there are increase in number of lipid droplets of small size, loss of glycogen which is present even after 3 hours. After 12 to 24 hours, the most conspicious changes are increased shrunken liver cells having high electron density. The cell organellae within these cells seem to be rather well preserved, although it is quite difficult to recognize in electron dense cell body.
The most noteworthy changes found in this series of experiment are lack of crystolysis or crystorrhexis, increase in electron density of ground substance and widening of middle pale layer of crista, of mitochondria. All of the biological meanings of these changes will be discussed in future.

3. Experiments now in progess.

a. Acute poisoning of Cl. Welchii toxin.

Purpose: To find the effects of Lecithinase and DNA-ase contained in the toxin on cells.
Liver was embbeded in epoxy resin and styrene for electronmicroscopy, and light optical preparations were finished.

b. Temborary ligation of renal artery.

Serial preliminary experiments revealed light optically that: 1) anoxic changes of tubular cells were observed clearly after

ligation of renal artery for 45 minutes which increase with lapse of time. But there is no anemic infarction. 2) Temporary ligation of renal artery for more than 30 minutes results in much more marked changes after reopening of the renal artery. It consists of peripheral coagulation necrosis and central autolytic changes(Anemic infarction). These changes begins to appear 1 hour reopening of renal artery. In case of 30 minutes of ligation, the changes seen 1 hour after reopening are less conspicious. The typical anemic infarction develope in case of 2-3 hours ligation-1 hour after reopening or in case of 1 hour ligation-3 hours after reopening.

Experiments for taking electron microsconic specimen will be started in near future.

c. Diphteria toxin.

Specimen were embedded to be cut.